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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 16867-2PCT	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/CA 03/00617	International filing date ( <i>day/month/year</i> ) 24.04.2003	Priority date ( <i>day/month/year</i> ) 24.04.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/06		
Applicant BIOGENTIS INC. et al.		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of 5 sheets, including this cover sheet.  <input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  These annexes consist of a total of 5 sheets.
3.	This report contains indications relating to the following items: <ul style="list-style-type: none"> <li>I <input checked="" type="checkbox"/> Basis of the opinion</li> <li>II <input type="checkbox"/> Priority</li> <li>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li>IV <input type="checkbox"/> Lack of unity of invention</li> <li>V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li>VI <input type="checkbox"/> Certain documents cited</li> <li>VII <input type="checkbox"/> Certain defects in the international application</li> <li>VIII <input type="checkbox"/> Certain observations on the international application</li> </ul>

Date of submission of the demand  11.11.2003	Date of completion of this report  25.06.2004
Name and mailing address of the international preliminary examining authority:   European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer  Teyssier, B  Telephone No. +31 70 340-2062  

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/CA 03/00617

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1, 3-11 as originally filed  
2 received on 25.05.2004 with letter of 19.05.2004

**Claims, Numbers**

1-26 received on 25.05.2004 with letter of 19.05.2004

**Drawings, Sheets**

1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	3, 4, 12, 16, 17
	No: Claims	1, 2, 5-7, 9-11, 13-15, 18-26
Inventive step (IS)	Yes: Claims	16, 17
	No: Claims	1-15, 18-26
Industrial applicability (IA)	Yes: Claims	1-26
	No: Claims	none

2. Citations and explanations

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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**Re Item I**

*Basis of the report*

The amendments filed with the letter of 19 May 2004 are allowable under Article 34(2)(b) PCT. Although the language of amended claims 1 and 18 is not to be found *expressis verbis* in the application as filed, which e.g. refers to "edge fusion" (p. 6) rather than to "edge assembling", it appears to be linguistically equivalent to the description given at pages 2, 3, 5 and 6.

**Re Item V**

*Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement*

Reference is made to the following documents:

- D1 L'Heureux et al., *Faseb Journal*, 1998, 12, 47-57 (January 1998)
- D2 US 6,133,030 A (General Hospital Corporation; Massachusetts Institute of Technology) 17 October 2000
- D3 WO 00/66036 A (Massachusetts General Hospital) 9 November 2000

D1 describes a process for making artificial blood vessel constructs by wrapping sheets of vascular smooth muscle cells and fibroblasts around a tubular support. As far as the tissue construct of claim 1 is a blood vessel, as provided by the example of the application, it is not new over the vessel constructs of D1, the subject-matter of claims 18-26 therefore lacks novelty (Article 33(2) PCT). Although the process of construction of the blood vessel in D1 is different from the process of the invention in that no edge assembling of sheets occurs, the final products obtained by each process do not appear different, or, if any relevant difference exist in the products, said difference is not reflected in the claims other than by reference to the production process.

D2 describes the co-cultivation of cells on micro-patterned supports for the purpose of regulating metabolic and synthetic functions; the patterned support allows for edge contact between different cell populations and fusion of the cell populations into one continuous, patterned, sheet. Part II of D2 specifically describes the construction of artificial liver constructs with hepatocytes grown as continuous populations over designated parts of the support and fibroblasts grown on the remaining parts of the support. Part III compares this setting with co-cultures in which cells are separated by removable polymer annuli, which allow for edge contact between the two-dimensional cell populations. While no examples are given, the use of other cell types is considered, including endothelial cells and smooth muscle cells (col. 2, point c). In view of Parts II and III of D2, the subject-matter of claims 1, 2, 5-7, 9-11, 13-15 and 18-26 is not new (Article 33(2) PCT) and the additional subject-matter of claims 3, 4, 8 and 12 does not involve an inventive step over D2 (Article 33(3) PCT).

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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D3 describes a process for making three-dimensional vascularised organs by growing a branching pattern of vascular cells on a two-dimensional mould, applying a sheet of cells (in the example, hepatocytes) to the resulting vascular structure and eventually rolling the vascularised cell sheet into a three-dimensional cylinder. Thus, the process foresees overlapping of the cell populations rather than edge contact and yields solid three-dimensional tissue constructs vascularised by a network of small vessels rather than a single, hollow, construct such as a large blood vessel; D3 does not anticipate the subject-matter of the application.

None of the prior art documents describes a process of rolling a single tissue construct comprising different cell populations assembled by edge contact, the subject-matter of claims 16 and 17 is therefore new (Article 33(2) PCT).

With special respect to the specific embodiment of claim 17 and the example, the closest prior art is D1 and the problem of the application can be defined as the provision of an alternative method for producing a blood vessel; an inventive step can be acknowledged for the subject-matter of claims 16 and 17 (Article 33(3) PCT) as no suggestion can be found in the prior art documents to assemble the cell populations by edge contact before rolling one single continuous sheet into a tube rather than by wrapping different homogenous sheets onto each other, as in D1.

**WHAT IS CLAIMED IS:**

1. A method for producing a continuous living tissue construct comprising allowing edge contact of at least one cell population with edge of at least another separated cell population maintained in culture, each cell population forming a living tissue sheet, for a period of time sufficient for edge assembling of said cell populations into a single continuous living tissue construct.
2. The method of claim 1, wherein said cell populations are partially or totally confluent.
3. The method of claim 1, wherein said cell population are embedded into a gel before being placed in culture for allowing edge contact.
4. The method of claim 3, wherein said gel is a collagen gel.
5. The method of claim 1, wherein said cell populations are composed of homologous or heterologous cells.
6. The method of claim 1, wherein said cell populations are composed of mammalian cells.
7. The method of claim 1, wherein said cell populations are composed of cells selected from the group consisting of mesenchymal cells, muscle cells, or fibroblasts.
8. The method of claim 7, wherein said muscle cells are smooth muscle cells.

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9. The method of claim 1, wherein said cell populations comprise at least one type of cells.
10. The method of claim 1, wherein said living tissue is a sheet comprised of at least one cell layer.
11. The method of claim 1, wherein at least one of said cell populations is a single cell layer, a tri-dimensional tissue construct or a tissue graft.
12. The method of claim 1, wherein at least one of said cell populations comprises genetically transformed cells.
13. The method of claim 1, wherein said cell populations are separated by a separator.
14. The method of claim 13, wherein said separator is impermeable or allows selective passage of components contained in a culture medium.
15. The method of claim 1, wherein said contact is caused by removal of a separator between the at least two cell populations, or by placing said cell populations in contact.
16. A method for producing a tubular tissue construct comprising rolling the continuous tissue construct of claim 1.
17. The method of claim 16, wherein said tubular tissue construct is a blood vessel.

18. A single continuous tissue construct comprising at least two edge to edge assembled cell populations forming living tissue sheets as produced by the method of claim 1.

19. The single continuous tissue construct of claim 18, wherein said living tissue sheets are composed of homologous or heterologous types of cells.

20. The single continuous tissue construct of claim 19, wherein said types of cells are mammalian cells.

21. The single continuous tissue construct of claim 19, wherein said types of cells are selected from the group consisting of mesenchymal cells, muscle cells, or fibroblasts.

22. The single continuous tissue construct of claim 21, wherein said muscle cells are smooth muscle cells.

23. The single continuous tissue construct of claim 18, wherein at least one living tissue sheet comprises at least one type of cells.

24. The single continuous tissue construct of claim 18, wherein said living tissue sheet comprises at least one cell layer.

25. The single continuous tissue construct of claim 18, wherein said living tissue sheets are placed in edge contact after removal of a separator to form said continuous tissue construct.

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26. The single continuous tissue construct of claim 25, wherein said separator is impermeable or allows selective passage of components contained in a culture medium in which are maintained said living tissue sheets.

eventually detach from the culturing substratum. This auto-assembly method yields tissue with organ-like mechanical properties and devoid of any synthetic material, making it perfectly compatible with the living organism in which it is to be implanted, as shown in L'Heureux et al. (FASEB J. 1998, 12:47-57).

5           When a tissue reconstructed with layers of different cell types has to be produced, the successive assembly of the different separated sheets increase the production time of the final product. Furthermore, tissue artifacts may appear at the point of fusion between the sheets as prepared in the art.

## 10    SUMMARY OF THE INVENTION

One object of the present invention is to provide a method for assembling living tissue sheets for forming a continuous tissue construct comprising the step of:

15           providing at least two cell populations capable of forming at least two separated living tissue sheets, the cell populations being partially or totally confluent; and

causing edge contact between the cell populations of step a) for a period of time sufficient for assembling the living tissue sheets into a single continuous tissue construct.

20           The cell populations of step a) may be composed of homologous or heterologous types of cells and are preferably mammalian cells selected from the group consisting of mesenchymal cells, muscle cells, smooth muscle cells or fibroblasts.

25           Another object of the present invention is to provide single continuous tissue construct composed of at least two living tissue sheets, wherein the living tissue sheets are placed in edge contact for a period of time